Molecular docking of novel, short peptide from *Flammulina velutipes* with influenza drug targets in comparison with anti-influenza drugs amantadine, oseltamivir and zanamivir

Abo Almaali, H. M.
Branch of Clinical Laboratory Sciences, College of Pharmacy, Kerbala University, Iraq.

Keywords: Influenza virus, Antiviral peptide, Molecular docking, Nuraminidase, Hemagglutinin

Received (Sebtember), **Accepted** (December)

ABSTRACT

Influenza viruses known to cause widespread morbidity and mortality, and distributed throughout several parts of the world, producing a serious public health threat, and may result in a pandemic. The antimicrobial peptides known to have broad spectrum activity and considered a promising agents in microbial inhibition and killing. So that this study was done to find the probable scope of anti-influenza activity of short peptide FV16 (KKVGTSKVVAKTVTKK) a sixteen amino acids short peptide inspired from the bacteria *F. velutipes*. Hex 8.0.0 software was used to find the probable binding positions and binding strength of FV16 with particular viral targets. This study was resulted in a high degree of binding strength of FV16 with the most studied influenza targets in comparison with the binding strength of other approved anti-influenza drugs (amantadine, oseltamivir and zanamivir), the influenza drug targets were neuraminidase, hemagglutinin, M1 and M2 proteins. Furthermore, FV16 binding positions on each particular viral targets derived from particular strain have its own unique position, this may give it the property of multi-strain binding agent to expand its spectrum against influenza virus strains. In conclusion, FV16 may have the potential to be an anti-influenza agent, and may bind to future generated strains. Future *in vitro* studies needed to prove this hypothesis.

التكامل الجزيئي لببتيد جديد وقصير مستل من بكتريا *Flammulina velutipes* مع اهداف دوائية في فايروس الانفلونزا مقارنة بالأدوية المضادة للأنفلونزا amantadine و oseltamivir و zanamivir

حسن محمود موسى ابو المعالي
فرع العلوم المختبرية السريرية، كلية الصيدلة، جامعة كربلاء، العراق.

الكلمات المفتاحية: فايروس الانفلونزا ،الببتيدات المضادة للفايروسات ،التكامل الجزيئي ، نيورامنيديز ، هيماكلوتينين

الخلاصة

يعد فايروس الانفلونزا من الجراثيم المنتشرة بشكل واسع ويسبب الكثير من الاصابات وقد تقود الاصابة ببعض السلالات الى الوفاة. و ان هذا الفايروس يتوزع في مناطق مختلفة من العالم مسبباً خطراً كبيراً على الصحة العامة وقد يصل الى حدوث الاوبئة المجتاحة. ان الببتيدات المضادة للجراثيم معروفة بكونها ذات مدى فعالية واسع وتعد عوامل واعدة في تثبيط وقتل الاحياء المجهرية .لذلك تم اجراء هذه الدراسة لإيجاد المدى المحتمل للارتباط بفايروس الإنفلونزا للببتيد القصير FV16 (KKVGTSKVVAKTVTKK) المتكون من 16 حامض اميني والمستل من بكتريا *F. velutipes*. تم استخدام برنامج Hex 8.0.0 لإيجاد مواضع الارتباط المحتملة لبعض الاهداف الدوائية في فايروس الانفلونزا مع هذا الببتيد وكذلك شدة الارتباط بينهما. نتج عن هذه الدراسة اكتشاف شدة ارتباط عالية بين ببتيد FV16 مع اغلب الاهداف الدوائية المدروسة، وشدة ارتباط هذه الاهداف الدوائية مع ببتيد FV16 كانت اعلى من ما هو حاصل فيما بين الاهداف الدوائية وبعض الادوية المقرة دولياً ضد فايروس الانفلونزا. وتتضمن هذه الاهداف الدوائيةكلاً من neuraminidase و

hemagglutinin و بروتينا M1 وM2. فضلا عن ذلك فان مواضع ارتباط ببتيد FV16 مع كل هدف معين مأخوذة من سلالات مختلفة تتباين حسب السلالة الفايروسية المدروسة ، و هذه الصفة قد تعطي ببتيد FV16 صفة القدرة على الارتباط بالسلالات المختلفة اي توسع الطيف في امكانية الارتباط بالإنفلونزا. ان نتائج الدراسة الحالية تدعو للاستنتاج ان ببتيد FV16 قد تكون له الامكانية لان يكون عامل مضاد للإنفلونزا ، وقد يعمل ايضا على السلالات المتولدة مستقبلا، وهنالك حاجة للعديد من الدراسات المختبرية الاخرى لإثبات هذه النظرية.

1. INTRODUCTION

The viral infections considered generally a serious life threat, and several of them causes several malicious diseases, as human immunodeficiency virus (HIV) and Avian Influenza, therefore, a novel therapeutics are needed to combat the viral infection [1]. The protein docking is the task of calculating the 3D structure of a protein complex from its model built units, to find a relatively low number of putative docking orientations, which may be refined and re-scored using more sophisticated techniques [2].

Cationic antimicrobial peptides (AMPs) and host defence peptides (HDPs) show the vast potential as peptide-based drugs. Considerable effort has been made in order to exploit their mechanisms of action, aiming to identify their targets, and to enhance their activity and bioavailability, the AMPs are natural compounds found in several organisms such as microorganisms, animals and plants [3].

These peptides can be classified according physical and chemical properties such as hydrophilic, amphipathic, cations and anions [4]. They are diverse in the length and sequence, two features are often the hallmarks of these molecules, they are cationic, approximately ranged from +2 to +7 at pH 7, and amphipathic, so that their geometry confers relatively polar hydrophilic and hydrophobic faces [5]. Despite their structural conservation, they have an extended spectrum of activity such as antibacterial [6], antioxidative [7], antifungal [8], antiviral [9], and antitumor [10].

This study was done to find the probable scope of anti-influenza activity of short peptide, through finding probable binding positions of particular viral target molecules and predicting their binding strength to the FV16, a sixteen amino acid peptide inspired from the bacteria *F. velutipes*.

2. MATERIALS AND METHODS

Materials

Antimicrobial peptide FV16 (KKVGTSKVVAKTVTKK) taken from a previous study [11], the viral proteins 3D structure was taken from RCSB Protein Data Bank as PDB format (http://www.rcsb.org/pdb/home/home.do), and the approved drugs were taken in SDF format from the PubChem compound database (http://www.ncbi.nlm.nih.gov/pccompound).

Methods

Molecular docking of receptors and ligands, was done using Hex 8.0.0 [12]. Cell penetrating probability was estimated using CellPPD (http://crdd.osdd.net/raghava/cellppd/index.html) [13]. Pymol (the PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC.) and chimera software were used for results visualization [14]. Immunogenicity was predicted using T cell epitope: Class I immunogenicity (http://tools.immuneepitope.org/immunogenicity/) [15]. ToxinPred used for the estimation of FV16 toxicity and chemical-physical properties (http://crdd.osdd.net/raghava/toxinpred/design.php)[16].

3. RESULTS

The short peptide FV16 (KKVGTSKVVAKTVTKK) have two pairs of positively charged amino acid lysine (K) found in both amino and carboxyl terminus, while some hydrophobic residues fall in the middle region (figure 1.A) . In addition, FV16 structure has both helical and random bents (figure 1.B). Polar residues (with glycine) equals 11, non-polar residues equals 5, uncharged residues (with glycine) equals 5 (serine= 1, thrionin= 3, glycin= 1), charged residues (only lysine)= 6, no hydrophobic face found, nonpolar residues equals 5, as shown in figure 2. Isoelectric point (pI) of FV16 equals 11, net charge at pH 7 equals +6 (figure 3).

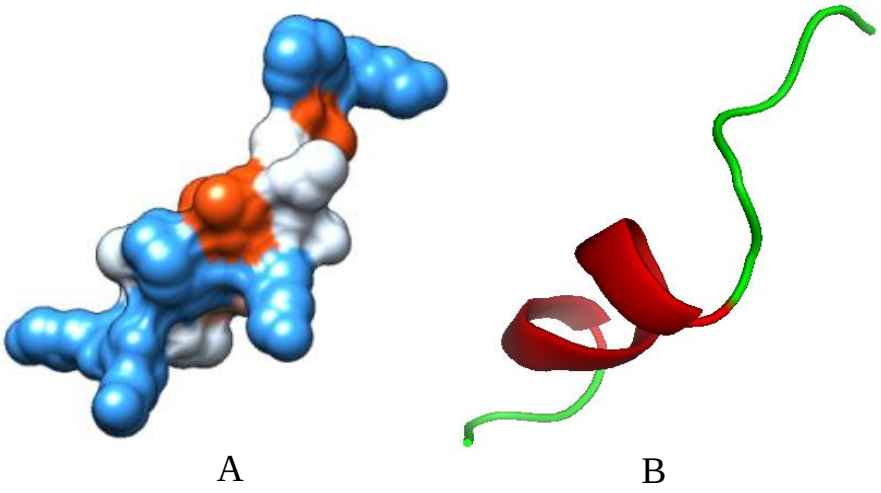

Figure 1: The *Flammulina velutipes* histone fragment (KKVGTSKVVAKTVTKK) FV16. A/ hydrophobicity surface, red is hydrophobic and blue is hydrophilic. B/ carton secondary structure, red is helix and green is random coil.

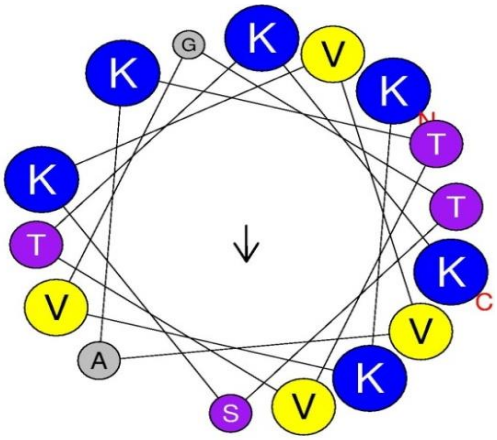

Figure 2: Physical-chemical properties of FV16.

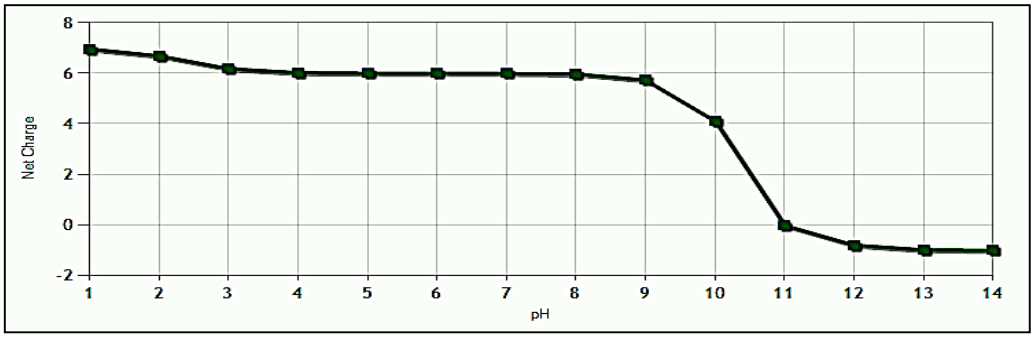

Figure 3: FV16 net charge versus pH, and isoelectric point.

Tables 1, 2, and 3 represent several influenza drug targets and their binding energy to FV16. The FV16 have a lower binding total energy for neuraminidase from several influenza virus strains in comparison with approved drugs used to treat influenza. As shown in the table 1 for influenza A, that ranged from -210.40 to -952.70 kcal/mol, except to influenza B virus neuraminidase with PDB id: 1NSD and 1NSC binding total energy were 0.0. In addition, FV16 docking with another influenza target hemagglutinin, resulted also in low total binding energy, table 2, in comparison with approved drugs, table 4. Other influenza virus targets, M1 and M2 proteins, as shown in table 3, also characterized by high total binding energy, in comparison to approved drugs, as shown in table 4.

Table 1: Biding energy of neuraminidase molecules taken from several strains to FV16 (using Hex server)

No.	PDB id *	Description	Total energy
1	3B7E	Neuraminidase of A/Brevig Mission/1/1918 H1N1 strain in complex with zanamivir	-952.70
2	1F8B	Neuraminidase (Native influenza virus neuraminidase in complex with NEU5AC2EN)	-946.60
3	1V0Z	Structure of neuraminidase from English duck subtype N6	-861.70
4	4K1H	Induced opening of influenza virus neuraminidase N2 150-loop suggests an important role in inhibitor binding	-686.30
5	3BEQ	Neuraminidase of A/Brevig Mission/1/1918 H1N1 strain	-681.10
6	3SAL	Crystal structure of Influenza A Virus Neuraminidase N5	-680.80
7	3K36	Crystal structure of B/Perth Neuraminidase	-666.00
8	3CYE	Cyrstal structure of the native 1918 H1N1 neuraminidase from a crystal with lattice-translocation defects	-660.90
9	4K1J	Induced opening of influenza virus neuraminidase N2 150-loop suggests an important role in inhibitor binding	-644.90
10	2QWA	X-ray structure of adrug resistante variant R292K of TERN N9 influenza virus nuraminidase	-633.84
11	4B7J	H1N1 2009 pandemic Influenza Virus: Resistance of the I223R Neuraminidase Mutant Explained by Kinetic and Structural Analysis	-601.60
12	2HT5	N8 Neuraminidase	-588.70
13	4HZV	The crystal structure of influenza A neuraminidase N3	-580.80
14	4HZY	Neuraminidase N3-H274Y (Influenza A)	-576.36
15	4MWL	Shanghai N9 distinct neuraminidases from avian-origin human-infecting H7N9 influenza viruses	-550.90

16	3NSS	Neuraminidase (2009 pandemic H1N1 neuraminidase N1 lacks the 150-cavity in its active sites)	-497.40
17	1NN2	Neuraminidase (H5N1)	-324.24
18	2HTY	N1 neuraminidase	-274.90
19	2HTV	N4 neuraminidase	-210.40
20	1NSD	Influenza B virus neuraminidase	0.00
21	1NSC	Influenza B virus neuraminidase	0.00

* PDB id = protein data bank database identification code.

Table 2: Biding energy of hemagglutinin molecules taken from several strains to FV16 (using Hex server)

No.	PDB id	Description	Total energy
1	2IBX	Influenza virus (VN1194) H5 HA	-1726.70
2	1RUZ	Hemagglutinin 1918 H1	-1418.10
3	2WR0	Hemagglutini H2 influenza	-1154.80
4	2FK0	Hemagglutinin influenza virus H5N1	-1151.80
5	4BSA	Haemagglutinin (with Asn-133 Glycosylation) from an H7N9 influenza virus isolated from humans	-1145.20
6	4LN6	Hemagglutinin from a H7N9 influenza virus (a/shanghai/2/2013)	-1121.40
7	3S11	Hemagglutinin H5N1 influenza virus, strain 437-10	-1117.60
8	1MQL	Hemagglutinin of a potential H3 avian progenitor of the 1968 Hong Kong pandemic influenza virus.	-1102.00
9	1MQM	Hemagglutinin of a potential H3 avian progenitor of the 1968 Hong Kong pandemic influenza virus	-1076.30
10	1TI8	H7 Haemagglutinin	-1050.10
11	1JSD	Haemagglutinin H9 SWINE	-1016.10
12	1RD8	Hemagglutinin H1 Precursor (HA0) 1918 Human	-946.20
13	2VIU	Haemagglutinin	-940.20
14	1RUY	Hemagglutinin H1 Swine1930	-886.20
15	4LCX	Hemagglutinin from avian-origin H7N9 influenza virus (A/Shanghai/1/2013)	-885.70
16	1HA0	Haemagglutinin PRECURSOR HA0	-832.70
17	4LXV	Hemagglutinin from a H1N1pdm A/WASHINGTON/5/2011 virus	-737.20
18	1RU7	Hemagglutinin H1 Human 1934	-685.70
19	4M4Y	Hemagglutinin 2009 H1N1 influenza virus with a stabilization mutation HA2 E47G	-684.80
20	2YP7	Haemagglutinin of 2005 Human H3N2 Virus	-624.60
21	2YP2	Haemagglutinin of 2004 Human H3N2 Virus	-622.20
22	4LKI	Hemagglutinin L226Q mutant from a avian-origin H7N9 influenza virus (A/Anhui/1/2013)	-510.80
23	4KOL	Hemagglutinin from avian-origin H7N9 influenza virus	-440.60

Table 3: Biding energy of M1 and M2 proteins molecules to FV16 (using Hex server)

No.	PDB id	Description	Total energy
1	1EA3	M1 protein	-720.20
2	3BKD	M2 protein (Transmembrane domain of M2 protein of Influenza A)	-610.34

Table 4: Anti-influenza drugs binding energy to corresponding targets (using Hex server)

Drug	Mode of action	Target	PDB	Description	**Total energy**
Amantadine	interferes with viral uncoating	Influenza A	3BKD	Transmembrane domain of M2 protein	-139.16
Oseltamivir	homolog of sialic acid	Influenza H5N1	1NN2	Neuraminidase	-253.20
Zanamivir	neuraminidase inhibitor	Influenza A	1V0Z	Neuraminidase	-258.06

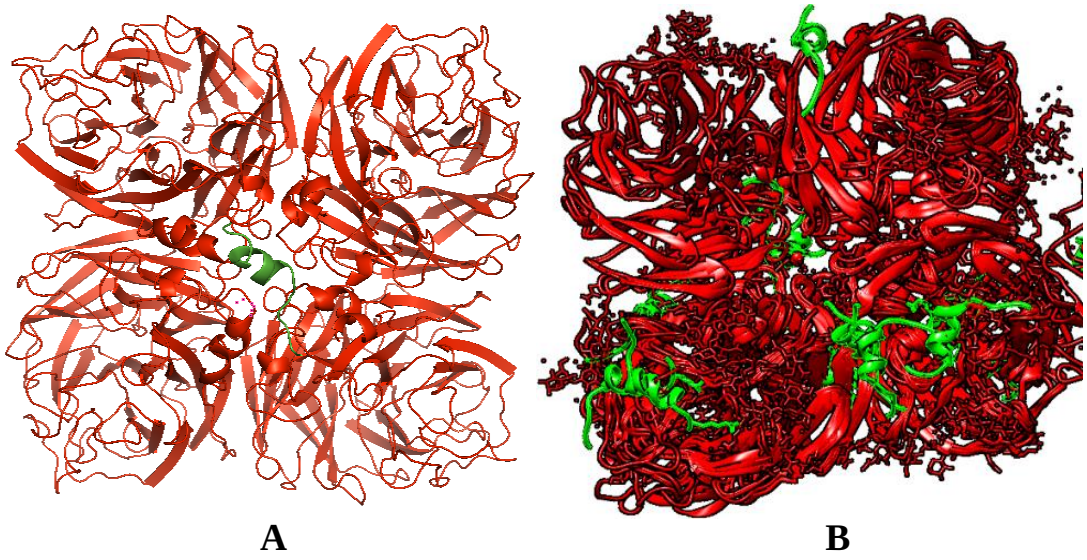

Figure 4: A/ red, influenza neuraminidase from English duck (PDB: 1V0Z) in complex with FV16, green. B/ red, superimposition of several neuraminidase molecules in complex with corresponding FV16 peptides, showed different binding positions of FV16 to each particular neuraminidase molecule.

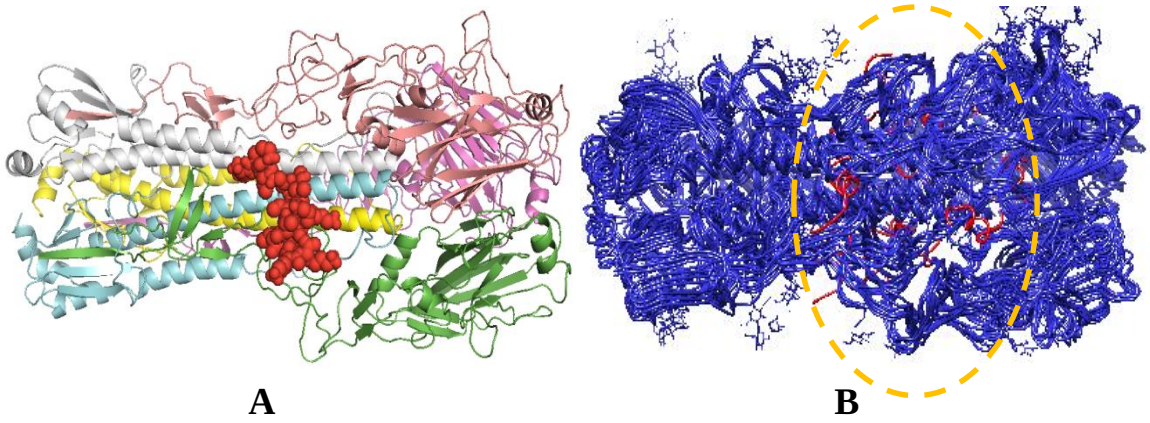

Figure 5: A/ influenza hemagglutinin from avian-origin H7N9 influenza virus (A/Shanghai/1/2013) (PDB: 4CLX) in complex with FV16 (red). B/ (blue) superimposed hemagglutinin molecules of the strains listed in the table (2) with their corresponding attachment positions to FV16 (red).

Table 5: FV16 anti-cancer activity prediction using anti-CP and toxicity using ToxinPred

Anti-cancer prediction		Toxicity prediction	
SVM score	Prediction	SVM score	Prediction
0.72	Anticancer	-1.31	Non-Toxin

Table 6: T cell epitope :Class I Immunogenicity

Peptide	Length	Score
KKVGTSKVVAKTVTKK	16	-0.39388

Table 7: Prediction of FV16 cell penetration by CellPPD

Peptide	SVM score	Prediction	Steric hindrance	Sidebulk
KKVGTSKVVAKTVTKK	0.02	Cell penetrating peptide	0.64	0.64

Table 8: Estimation of FV16 chemical-physical properites

Hydrophobicity	Hydropathicity	Hydrophilicity	Amphipathecity	Charge	Mol wt
-0.30	-0.51	0.66	1.38	+6.00	1702.35

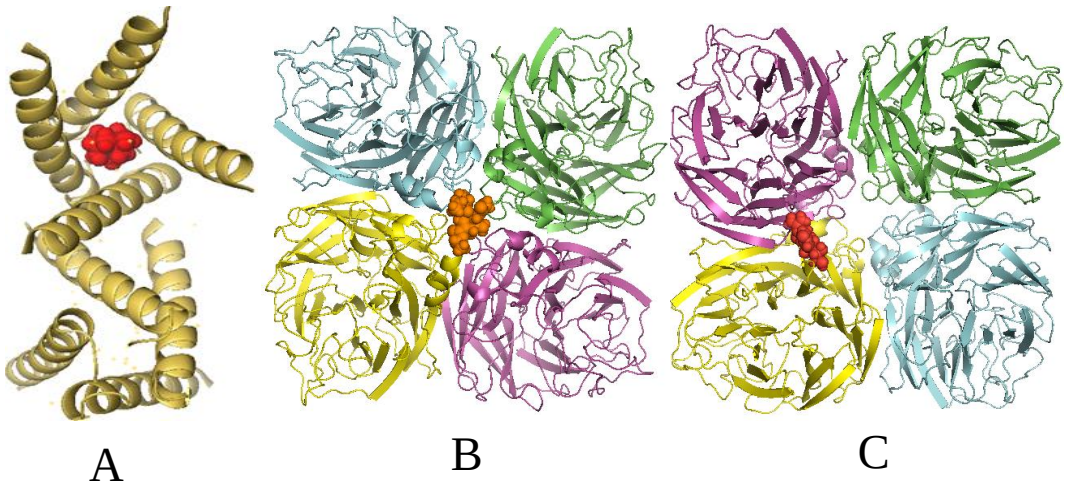

Figure 6: A: Amantadine in complex with transmembrane domain of M2 protein (PDB: 3BKD), B: oseltamivir (Tamiflu) in complex with nuraminidase (PDB: 1NN2). C: Zanamivir in complex with nuraminidase (1VOZ).

4. DISSCUSION

The influenza virus has caused several seasonal influenza epidemics and pandemics, which resulted in a serious threat to public health and socioeconomic impacts, in particular cases may lead to death [17]. Vaccine treatment is useless for controlling influenza infection, this happens because of the occurrence of mutations in the influenza virus continuously, changing drug targets conformation and other physicochemical properties, led to increase its

resistance status. Some influenza virus strains are also resistant to some antiviral drugs like oseltamivir and zanamivir, which inhibit neuraminidase. Another solution for controlling this virus is to find a new design for antiviral drugs [18].

From a previous study, the bioinformatic approaches led to suggestion of FV16 (KKVGTSKVVAKTVTKK) as a molecule with antiviral activity [11], due to limited researches concerning this molecule, and the scope of its action against numerous influenza targets still in need to be evaluated, this is done using another bioinformatic approach, the molecular docking.

Usually antimicrobial peptides characterized with positive net charge [19], as for FV16 equals +6, also the distribution of lysine, as two residues in both amino and carboxy terminals may enhance the stability of receptor ligand complex (FV16–influenza target), as microbial charge usually negative [20]. In addition, the isoelectric point of FV16= 11, which ensure its solubility in human and animal's pH [21], as shown in figure 3.

In addition, the nuraminidase the enzyme that enables the virus to be released from the host cell, by cleaving sialic acid groups from glycoproteins, and required for influenza virus replication [22]. In the current study, the molecular docking of FV16 with neuraminidase molecules from 21 different influenza strains reviled strong binding energy ranged from (-952.70 to -210.40 kcal/mol), with the zero binding energy of influenza B nuraminidase molecules with PDB ID, 1NSD and 1NSC, as shown in table 1. This may inhibit the nuraminidase enzyme activity and lead to block a part of the influenza virus life cycle.

On the other hand, Hemagglutinin, the glycoprotein of the viral envelope, which plays a critical role in the viral binding, fusion and entry [23]. Therefore, HA is an attractive target for developing anti- influenza drugs to block the entry step of infection. The conformational changes of HA during viral fusion process and the development of HA-based IAV entry inhibitors, may provide a new choice for controlling future influenza pandemics. For FV16, molecular docking with 23 different hemagglutinin molecules from different influenza strains were ranged from -1726.70 to -440.60 kcal/mol. This suggests FV16 as a new probable antiviral agent.

Furthermore, the M1 and M2 is a multifunctional protein that plays an essential structural and functional role in the virus life cycle, through driving the virus budding and is the major protein component of the virion. Where it forms an intermediate layer between the viral envelope and integral membrane proteins and the genomic ribonucleoproteins [24]. In the current study, the binding energy of M1 and M2 proteins molecules to FV16 were -720.20 and -610.34 kcal/mol, respectively, this supports the suggestion of FV16 as a new probable antiviral agent.

Finally, the approved anti-influenza drugs binding energy to corresponding targets were -258.06 for amantadine, -253.20 for Oseltamivir and -139.16 for zanamivir, as shown in table 4. From that, the binding energies of the most studied influenza targets with FV16 were stronger than some approved anti-influenza drugs, and in certain cases, several times stronger, subjecting them theoretically being an a broad spectrum anti influenza agents.

The position of FV16 binding to particular influenza virus targets are varied according to strain from which this target is derived, as in case of nuraminidase, multiple binding positions were found because of strain differences, and distributed in all molecule's parts, as shown in figure 4. While, for hemagglutinin molecule, FV16 have several binding positions aggregated in one part of the molecule, as shown in figure 5. In addition, in particular cases, an approved anti-influenza drug like oseltamivir (tamiflu) and zanamivir bind to

nuraminidase in a similar topological manner to that of FV16 binding to it, as shown in figure 6.

On the other hand, FV16 prediction as anticancer agent using anti-CP algorithm revealed an acceptable score to be anti-cancer equals 0.72. In addition the prediction of FV16 toxicity using ToxinPred resulted in no probable toxicity. Furthermore, the low molecular weight, low complexity, and amino acids composition of FV16 are several factors may help it to escape host immune system, and this agrees with the immunogenicity prediction score, which was negative to be T cell epitope, as shown in table 6.

Several physicochemical properties of FV16, as shown in tables 7 and 8, give this protein the ability to penetrate cells and be soluble in both water and lipids, these characters are important for this short peptide if used in medical applications.

5. CONCLUSION

Based on simulation results, the molecular docking demonstrated for studied influenza targets have a chance to bind to the short peptide FV16, in addition to its ability to be distributed in biological fluids, this may subject it as a potent anti-influenza agent *in vivo*. In addition, this short peptide has a chance to act against new strains. Several studies are needed to prove these theories both in vivo and in vitro.

ACKNOWLEDGEMENTS

Author is thankful to Prof. Dr. Zahra Al-Khafaji and Dr. Adel Alnasrawy for sharing their knowledge, and all who participates in this work.

REFERENCES

[1] Tambunan US and Parikesit AA., (2012) HPV Bioinformatics: In silico detection, drug design and prevention agent development, university of Indonesia, InTech publisher, Indonesia, p. 237-252.

[2] Bachar O, Fischer D, Nussinov R, Wolfson HJ. (1993) A computer vision based technique for 3D sequence-independent structural comparison of proteins, Protein Eng. 6(3):279–288.

[3] Hancock RE, Sahl HG., (2006) Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies, Nat. Biotechnol., 24(12):1551–1557.

[4] Peter BM, Shirtliff ME, Jabra-Rizk MA., (2010) Antimicrobial peptides: primeval molecules or future drugs?, Plos Pathog., 6; e1001067.

[5] Nguyen LT, Haney EF and Vogel HJ., (2011) The expanding scope of antimicrobial peptide structures and their modes of action, Trends Biotechnol., 29 (9):464–472.

[6] Okubo BM, Silva ON, Migliolo L, Gomes DG, Porto WF, Batista CL, et al. (2012) Evaluation of an antimicrobial L-amino acid oxidase and peptide derivatives from Bothropoides mattogrosensis pitviper venom, PLoS ONE, 7:e33639.

[7] Power O, Jakeman P, Fitzgerald RJ, (2013) Antioxidative peptides: enzymatic production, in vitro and in vivo antioxidant activity and potential applications of milk-derived antioxidative peptides, Amino Acids, 44(3): 797–820.

[8] Mandal SM, Porto WF, Dey P, Maiti MK, Ghosh AK, Franco OL, (2013) The attack of the phytopathogens and the trumpet solo: identification of a novel plant antifungal peptide with distinct fold and disulfide bond pattern, Biochimie. 95 (10):1939–1948.

[9] Findlay EG, Currie SM, Davidson DJ, (2013) Cationic host defence peptides: potential as antiviral therapeutics, BioDrugs, 27(5):479–493.

[10] Silva ON, Porto WF, Migliolo L, Mandal SM, Gomes DG, Holanda HH, et al. (2012) Cn-AMP1: a new promiscuous peptide with potential for microbial infections treatment, **Biopolymers**, 98(4): 322–331.
[11] Abo Almaali HM, (2014) Antimicrobial peptides potential of some microbial histones: In-silico modeling, **Internation. J. Recent Scie. Res**., 5 (3):649-65.
[12] Ritchie D and Venkatraman V., (2010) Ultra-Fast FFT protein docking on graphics processors, **bioinformatics**, 26(19): 2398-2405.
[13] Gautam A, Chaudhary K, Kumar R, Sharma A, Kapoor P and Tyagi A, et al. (2013) In silico approaches for designing highly effective cell penetrating peptides, **J. Translational Med**., 11:74.
[14] Huang CC, Couch GS, Pettersen EF, and Ferrin TE., (1996) Chimera: an extensible molecular modeling application constructed using standard components, **Pacific Symposium on Biocomputing**, 1:724.
[15] Calis JJA, Maybeno M, Greenbaum JA, Weiskopf D, De Silva AD, Sette A, et al., (2013) Properties of MHC class I presented peptides that enhance immunogenicity. **PloS Comp. Biol**., 9(10):e1003266.
[16] Gupta S, Kapoor P, Chaudhary K, Gautam A, Kumar R, et al. (2013) In silico approach for predicting toxicity of peptides and proteins. **PLoS ONE**, 8(9):e73957.
[17] Yang J, Li M, Shen X, and LiuS, (2013) Influenza A virus entry inhibitors targeting the hemagglutinin, **Viruses**, 5(1): 352-373.
[18] Tambunan US, Amri N and Parikesit AA, (2012) In silico design of cyclic peptides as influenza virus, a subtype H1N1 neuraminidase inhibitor, **African J. Biotechnol**., 11(52): 11474-11491.
[19] Chaithanya ER, Philip R, Sathyan N, and Anil Kumar PR, ()2013 Molecular characterization and phylogenetic analysis of a histone-derived antimicrobial peptide teleostin from the marine teleost fishes, *Tachysurus jella* and Cynoglossus, **ISRN Molec. Biol.**, Article ID 185807, 7.
[20] Bahar AA, and Ren D, Antimicrobial peptides, (2013) **Pharmaceuticals** (Basel). 6(12): 1543–1575.
[21] Arnett TR, (2008) Extracellular pH regulates bone cell function, **J. Nutr**. 138(2):415S-418S.
[22] Zhu X, McBride R, Nycholat CM, Yu W, Paulson JC and Wilson IA., (2012) Influenza virus neuraminidase with reduced enzymatic activity that avidly bind sialic acid receptors, **J. Virol**. 86(24):13371-13383.
[23] McCullough C, Wang M, Rong L, Caffrey M, (2012) Characterization of influenza hemagglutinin interactions with receptor by NMR, **PLoS ONE**, 7(7):e33958.
[24] Noton SL, Medcalf E, Fisher D, Mullin AE, Elton D and Digard P, () Identification of the domains of the influenza A virus M1 matrix protein required for NP binding, oligomerization and incorporation into virions, **J. Gen. Virol**., 2007 88(8): 2280–2290.